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## RAW SEQUENCE LISTING

DATE: 07/08/2002

PATENT APPLICATION: US/10/033,325

TIME: 11:12:54

Input Set : A:\GC502-2-C1-SEQLIST.txt

Output Set: N:\CRF3\07082002\J033325.raw

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3 <110> APPLICANT: Poulouse, Ayrookaran J.
4   Schellenberger, Volker
5   Kellis, Jr., James T.
6   Paech, Christian
7   Nadherny, Joanne
8   Naki, Donald P.
9   Collier, Katherine D.
10  Caldwell, Robert M.
11  Baeck, Andre C.
13 <120> TITLE OF INVENTION: Multiply-Substituted Protease Variants
15 <130> FILE REFERENCE: GC502-2-C1
17 <140> CURRENT APPLICATION NUMBER: US 10/033,325
18 <141> CURRENT FILING DATE: 2001-11-02
20 <150> PRIOR APPLICATION NUMBER: US 09/178,155
21 <151> PRIOR FILING DATE: 1998-10-23
23 <150> PRIOR APPLICATION NUMBER: US 08/956,323
24 <151> PRIOR FILING DATE: 1997-10-23
26 <150> PRIOR APPLICATION NUMBER: US 08/956,564
27 <151> PRIOR FILING DATE: 1997-10-23
29 <150> PRIOR APPLICATION NUMBER: US 08/956,324
30 <151> PRIOR FILING DATE: 1997-10-23
32 <160> NUMBER OF SEQ ID NOS: 6
34 <170> SOFTWARE: FastSEQ for Windows Version 3.0
36 <210> SEQ ID NO: 1
37 <211> LENGTH: 1497
38 <212> TYPE: DNA
39 <213> ORGANISM: Bacillus amyloliquefaciens
41 <220> FEATURE:
42 <221> NAME/KEY: CDS
43 <222> LOCATION: (96)...(1245)
45 <400> SEQUENCE: 1
46 ggtctactaa aatattattc catactatac aattaatata cagaataatc tgtctattgg      60
47 ttattctgca aatgaaaaaa aggagaggat aaaga gtg aga ggc aaa aaa gta      113
48                               Met Arg Gly Lys Lys Val
49                               1           5
51 tgg atc agt ttg ctg ttt gct tta gcg tta atc ttt acg atg gcg ttc      161
52 Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe
53          10           15           20
55 ggc agc aca tcc tct gcc cag gcg gca ggg aaa tca aac ggg gaa aag      209
56 Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys
57          25           30           35
59 aaa tat att gtc ggg ttt aaa cag aca atg agc acg atg agc gcc gct      257
60 Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met Ser Ala Ala

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|     |   |      |    |  |
|-----|---|------|----|--|
| 61  | 40  | 45   | 50 |  |
| 63  | aag aag aaa gat gtc att tct gaa aaa ggc ggg aaa gtg caa aag caa | 305  |    |  |
| 64  | Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Gly Lys Val Gln Lys Gln |      |    |  |
| 65  | 55 60 65 70   |      |    |  |
| 67  | ttc aaa tat gta gac gca gct tca gtc aca tta aac gaa aaa gct gta | 353  |    |  |
| 68  | Phe Lys Tyr Val Asp Ala Ala Ser Val Thr Leu Asn Glu Lys Ala Val |      |    |  |
| 69  | 75 80 85  |      |    |  |
| 71  | aaa gaa ttg aaa aaa gac ccg agc gtc gct tac gtt gaa gaa gat cac | 401  |    |  |
| 72  | Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp His |      |    |  |
| 73  | 90 95 100   |      |    |  |
| 75  | gta gca cat gcg tac gcg cag tcc gtg cct tac ggc gta tca caa att | 449  |    |  |
| 76  | Val Ala His Ala Tyr Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile |      |    |  |
| 77  | 105 110 115   |      |    |  |
| 79  | aaa gcc cct gct ctg cac tct caa ggc tac act gga tca aat gtt aaa | 497  |    |  |
| 80  | Lys Ala Pro Ala Leu His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys |      |    |  |
| 81  | 120 125 130   |      |    |  |
| 83  | gta gcg gtt atc gac agc ggt atc gat tct tct cat cct gat tta aag | 545  |    |  |
| 84  | Val Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys |      |    |  |
| 85  | 135 140 145 150   |      |    |  |
| 87  | gta gca agc gga gcc agc atg gtt cct tct gaa aca aat cct ttc caa | 593  |    |  |
| 88  | Val Ala Ser Gly Ala Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln |      |    |  |
| 89  | 155 160 165   |      |    |  |
| 91  | gac aac aac tct cac gga act cac gtt gcc ggc aca gtt gcg gct ctt | 641  |    |  |
| 92  | Asp Asn Asn Ser His Gly Thr His Val Ala Gly Thr Val Ala Ala Leu |      |    |  |
| 93  | 170 175 180   |      |    |  |
| 95  | aat aac tca atc ggt gta tta ggc gtt gcg cca agc gca tca ctt tac | 689  |    |  |
| 96  | Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr |      |    |  |
| 97  | 185 190 195   |      |    |  |
| 99  | gct gta aaa gtt ctc ggt gct gac ggt tcc ggc caa tac agc tgg atc | 737  |    |  |
| 100 | Ala Val Lys Val Leu Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile |      |    |  |
| 101 | 200 205 210   |      |    |  |
| 103 | att aac gga atc gag tgg gcg atc gca aac aat atg gac gtt att aac | 785  |    |  |
| 104 | Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn |      |    |  |
| 105 | 215 220 225 230   |      |    |  |
| 107 | atg agc ctc ggc gga cct tct ggt tct gct gct tta aaa gcg gca gtt | 833  |    |  |
| 108 | Met Ser Leu Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val |      |    |  |
| 109 | 235 240 245   |      |    |  |
| 111 | gat aaa gcc gtt gca tcc ggc gtc gta gtc gtt gcg gca gcc ggt aac | 881  |    |  |
| 112 | Asp Lys Ala Val Ala Ser Gly Val Val Val Val Ala Ala Ala Gly Asn |      |    |  |
| 113 | 250 255 260   |      |    |  |
| 115 | gaa ggc act tcc ggc agc tca agc aca gtg ggc tac cct ggt aaa tac | 929  |    |  |
| 116 | Glu Gly Thr Ser Gly Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr |      |    |  |
| 117 | 265 270 275   |      |    |  |
| 119 | cct tct gtc att gca gta ggc gct gtt gac agc agc aac caa aga gca | 977  |    |  |
| 120 | Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala |      |    |  |
| 121 | 280 285 290   |      |    |  |
| 123 | tct ttc tca agc gta gga cct gag ctt gat gtc atg gca cct ggc gta | 1025 |    |  |
| 124 | Ser Phe Ser Ser Val Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val |      |    |  |
| 125 | 295 300 305 310   |      |    |  |

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Input Set : A:\GC502-2-C1-SEQLIST.txt

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127 tct atc caa agc acg ctt cct gga aac aaa tac ggg gcg tac aac ggt      1073
128 Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly
129           315           320           325
131 acg tca atg gca tct ccg cac gtt gcc gga gcg gct gct ttg att ctt      1121
132 Thr Ser Met Ala Ser Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu
133           330           335           340
135 tct aag cac ccg aac tgg aca aac act caa gtc cgc agc agt tta gaa      1169
136 Ser Lys His Pro Asn Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu
137           345           350           355
139 aac acc act aca aaa ctt ggt gat tct ttg tac tat gga aaa ggg ctg      1217
140 Asn Thr Thr Thr Lys Leu Gly Asp Ser Leu Tyr Tyr Gly Lys Gly Leu
141           360           365           370
143 atc aac gta caa gcg gca gct cag taa a acataaaaaa ccggccttgg      1265
144 Ile Asn Val Gln Ala Ala Ala Gln *
145 375           380
147 cccgcgcggt tttttattat tttttcttct ccgcatgttc aatccgctcc ataatcgacg      1325
148 gatggctccc tctgaaaatt ttaacgagaa acggcggtt gacccggctc agtcccgtaa      1385
149 cggccaactc ctgaaacgtc tcaatcgccg cttcccggtt tccggtcagc tcaatgccat      1445
150 aacggtcggc ggcgttttcc tgataccggg agacggcatt cgtaatcgga tc      1497
152 <210> SEQ ID NO: 2
153 <211> LENGTH: 382
154 <212> TYPE: PRT
155 <213> ORGANISM: Bacillus amyloliquefaciens
157 <400> SEQUENCE: 2
158 Met Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu
159 1           5           10           15
160 Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly
161           20           25           30
162 Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met
163           35           40           45
164 Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly
165           50           55           60
166 Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Val Thr
167           65           70           75           80
168 Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala
169           85           90           95
170 Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro
171           100          105          110
172 Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr
173           115          120          125
174 Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser
175           130          135          140
176 Ser His Pro Asp Leu Lys Val Ala Ser Gly Ala Ser Met Val Pro Ser
177           145          150          155          160
178 Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala
179           165          170          175
180 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala
181           180          185          190
182 Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu Gly Ala Asp Gly Ser

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```

183          195          200          205
184 Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn
185          210          215          220
186 Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala
187 225          230          235          240
188 Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala Ser Gly Val Val Val
189          245          250          255
190 Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Ser Thr Val
191          260          265          270
192 Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp
193          275          280          285
194 Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu Asp
195          290          295          300
196 Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys
197 305          310          315          320
198 Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser Pro His Val Ala Gly
199          325          330          335
200 Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr Gln
201          340          345          350
202 Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys Leu Gly Asp Ser Leu
203          355          360          365
204 Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Ala Gln
205          370          375          380
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208 <211> LENGTH: 275
209 <212> TYPE: PRT
210 <213> ORGANISM: Bacillus amyloliquefaciens
212 <400> SEQUENCE: 3
213 Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
214 1          5          10          15
215 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
216          20          25          30
217 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
218          35          40          45
219 Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
220          50          55          60
221 Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
222 65          70          75          80
223 Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
224          85          90          95
225 Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
226          100          105          110
227 Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
228          115          120          125
229 Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
230          130          135          140
231 Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
232 145          150          155          160
233 Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala

```

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Input Set : A:\GC502-2-C1-SEQLIST.txt

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```

234          165          170          175
235 Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
236          180          185          190
237 Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
238          195          200          205
239 Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
240          210          215          220
241 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
242          225          230          235          240
243 Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
244          245          250          255
245 Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
246          260          265          270
247 Ala Ala Gln
248          275
250 <210> SEQ ID NO: 4
251 <211> LENGTH: 275
252 <212> TYPE: PRT
253 <213> ORGANISM: Bacillus subtilis
255 <400> SEQUENCE: 4
256 Ala Gln Ser Val Pro Tyr Gly Ile Ser Gln Ile Lys Ala Pro Ala Leu
257 1          5          10          15
258 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
259          20          25          30
260 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala
261          35          40          45
262 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His
263          50          55          60
264 Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly
265          65          70          75          80
266 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
267          85          90          95
268 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
269          100          105          110
270 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
271          115          120          125
272 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser
273          130          135          140
274 Ser Gly Ile Val Val Ala Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly
275          145          150          155          160
276 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala
277          165          170          175
278 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala
279          180          185          190
280 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
281          195          200          205
282 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr
283          210          215          220
284 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr

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VERIFICATION SUMMARY

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Input Set : A:\GC502-2-C1-SEQLIST.txt

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